DORMANT SEASON APPLICATION OF *STEINERNEMA CARPOCAPSAE* (RHABDITIDA: STEINERNEMATIDAE) AND *HETERORHABDITIS* SP. (RHABDITIDA: HETERORHABDITIDAE) ON ALMOND FOR CONTROL OF OVERWINTERING *AMYELOIS TRANSITELLA* AND *ANARSIA LINEATELLA* (LEPIDOPTERA: GELECHIIDAE)

FERNANDO AGUDELO-SILVA[1], FRANK G. ZALOM[2], ALBERT HOM[1],
AND LONNIE HENDRICKS[3]
Biosys, 1057 E. Meadow Circle
Palo Alto, CA 94303

[1]Current address: FAS Technologies. 1550 Walnut St. #5. Berkeley, CA 94709

[2]Department of Entomology, University of California, Davis, CA 95616

[3]Cooperative Extension, 2145 W. Wardrobe, Merced, CA 95340

ABSTRACT

Overwintering larval populations of *Amyelois transitella* in mummy almonds and *Anarsia lineatella* in hibernaculae on almond trees in California orchards were reduced by infectives of *Steinernema carpocapsae* strain All and a cold tolerant *Heterorhabditis* species (HL81) applied in the dormant season. The effect of this reduction in terms of preventing economic damage the following year is not known. Although the population reduction was statistically significant, it is unlikely the increase in mortality achieved would prove to be an economically viable alternative to current practices.

Key Words: Almond, *Steinernema carpocapsae*, *Heterorhabditis* sp., *Amyelois transitella*, *Anarsia lineatella*

RESUMEN

Poblaciones hibernanates de larvas de *Amyelois transitella* en “almendras momificadas”, y poblaciones hibernanates de *Anarsia lineatella* en hibernáculos de almendros en California, fueron reducidas por estados infectivos de dos nemátodos entomopatógenos, *Steinernema carpocapsae* cepa A11 y una especie de *Heterorhabditis* (HL81) tolerante a baja temperatura, aplicados durante la estación de dormancia. El efecto de esta reducción en términos de impedir el daño económico al año siguiente no es bien conocido. Aunque la reducción en la población fue estadísticamente significativa, se duda que el incremento de la mortalidad logrado sea una alternativa económicamente viable para las prácticas presentes de control de estos insectos.

For many years entomopathogenic nematodes have been considered to be potential biological mortality agents of many economically important insect pests (Glaser et al. 1940, Forschler & Gardner 1991). Because their nutritional requirements were poorly known for some time, it was difficult to produce adequate quantities of the nematodes for large scale field experiments. Advances in the understanding of the nutritional requirements (Buecher & Hansen 1977) and the development of mass culturing of these nematodes (Bedding 1984, Friedman 1990) has made it possible to produce them in sufficient quantities for such research. Increased emphasis on field evaluation will result in a better assessment of these organisms as practical insect control agents.

Entomopathogenic nematodes appear to have the greatest potential as control agents when directed against immature insect stages occupying secluded niches, such as split almonds (Lindegren et al. 1978, Lindegren et al. 1987), tree galleries (Deseo & Miller 1985), leaf sheaths and cabbage heads (Welch & Briand 1961), artichoke crowns (Bari & Kaya 1984) and soil crevices (Wright et al. 1987, Shanks & Agudelo-Silva 1990).

The use of entomopathogenic nematodes against two important insect pests of *Prunus* spp. that occupy secluded niches as larvae during the dormant season has not been fully investigated. These insects are the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) and the peach twig borer *Anarsia lineatella* (Zeller), Lepidoptera: Gelechiidae). Together, these insects are estimated to cause direct damage of more than $10 million to California almond growers alone, not including treatment costs. *A. transitella* is the most important insect pest of almonds. Larvae of the two summer generations invade the nuts following almond hullsplit and feed on the meats. Almond handlers accept up to 4% damaged meats, but they award bonuses to growers for lower damage (Klonsky et al. 1990). After harvest, some nuts remain on the trees where they continue to attract *A. transitella*. The nuts dry and become “mummies” which often contain *A. transitella* larvae. The overwintering larvae become adults the following spring and are the source of infestation in the subsequent crop. Cultural controls for *A. transitella* include removing these “mummies” and harvesting nuts as early as possible in the season to avoid peak moth flights. Almost half of the orchards (~ 100,000 hectares in a given year) receive in-season organophosphate sprays for *A. transitella* (Klonsky et al. 1990). While there is evidence that applications of beneficial nematodes can infect *A. transitella* larvae infesting newly split almonds in the summer (Agudelo-Silva et al. 1987, Lindegren et al. 1978), control of overwintering larvae has not been attempted.

A. lineatella is an important pest of both almonds and stone fruits in California, and the summer generation causes direct damage to nut meats and fruit. The larvae overwinter in hibernaculae built in crotches of twigs or branches. The larvae emerge during February and early March (Zalom et al. 1992) and migrate to shoot tips where they burrow into them to complete larval development. Organophosphate insecticides are routinely applied during the dormant season for *A. lineatella* control, and these have been the recommended alternative to disruptive in-season sprays (Rice & Jones 1988, Zalom et al. 1991). Recently, the application of dormant sprays has been criticized for environmental reasons, and suitable alternatives or mitigating measures are being pursued (e.g. Barnett et al. 1993).

Here we report results of field and laboratory trials employing two entomopathogenic nematode species applied during the dormant season for control of overwintering *A. transitella* and *A. lineatella* larvae in almonds. In the dormant season, pest populations are relatively low and concentrated on the mummy nuts.

Materials and Methods

Nematodes

Steinernema carpocapsae strain All and a cold tolerant *Heterorhabditis* species (currently undescribed but hereafter referred to as HL81) were used in the tests. *Het-*

erorhabditis sp. strain HL81 (isolated in Holland) has been shown to parasitize insects at 9°C and 10°C(Simmons & Van der Schaf 1986). Infective juveniles were artificially mass reared (Biosys unpubl.) and stored in moist foam at 5°C(*S. carpocapsae* strain All) or at 10°C (*Heterorhabditis* sp. strain HL81) until needed. Before each test application, the nematodes were examined under magnification to confirm that the survival rate was greater than 90 percent.

A. transitella

Trials were conducted in Le Grand, Merced Co., and Durham, Butte Co., California, during February and December, 1987, respectively. The Le Grand test was conducted to determine if mortality of overwintering *A. transitella* could be increased by a dormant season *S. carpocapsae* application. Treatments were applied on 23 February in a commercial orchard (var. "Le Grand") which was heavily infested with overwintering *A. transitella* (33% mummies infested in a pretreatment survey). Six applications rates were evaluated: 0, 0.62, 1.25, 2.5, 5.0, 10.0 and 20.0 million nematodes per tree. Each experimental unit was a tree and treatments were replicated five times. A totally randomized design was utilized. Nematodes were applied suspended in water (10.2 liters per tree) using a handgun sprayer (Farmtec, Oakland, CA). Immediately after the application, ten mummies were collected from each treated tree, individually placed in plastic bags in ice chests containing ice and brought to the laboratory to be examined for presence of nematodes. Nematodes were extracted from the mummies following the method developed by Lindegren et al (1987). One week after the application, 50 mummies were randomly collected from each of the trees, cracked by hand and examined for *A. transitella*. Larvae were tallied as alive or dead. Dead larvae were dissected and examined microscopically to ascertain the presence of nematodes.

The Durham test was conducted to compare the ability of both *S. carpocapsae* and *Heterorhabditis* sp. strain HL81 to infect *A. transitella* larvae at low temperature. Treatments were applied on 15 December in a commercial orchard (var. "Nonpareil", "Ne-plus Ultra," and "Mission"; 2:1:1 ratio).

Treatments evaluated were *S. carpocapsae*, *Heterorhabditis* sp. strain HL81 and an untreated control. Mummies were randomly collected from trees and divided into 15 groups each of which contained 130 mummies. The mummies were treated such that there was certainty that all the mummies received nematodes. Each group of mummies was placed on a piece of flat plastic laid out on the ground in the orchard and then arranged on the plastic touching each other, in a single layer. Twenty mummies were taken from each group and examined for *A. transitella* larvae. Infestation of these mummies was determined to be 59.5%. Each group was randomly assigned to one of the treatments, and the treatments were applied using a previously calibrated spray bottle. Nematodes were suspended in water and applied at a rate of 51 nematodes per mummy (5,610 nematodes suspended in water were applied to each group of 110 mummies). Immediately after application (less than 15 minutes), 10 mummies were randomly chosen from each group, placed in individual bags and stored in ice chests to be brought to the laboratory for nematode extraction (Lindegren et al.1987). The remaining mummies (100 per group) were placed in individual wire mesh cages which were suspended from branches in the almond trees. Two weeks after the nematodes were applied, the mummies from each of the cages were placed in ice chests containing ice and brought to the laboratory to be examined for the presence of larvae. Dead larvae were dissected and microscopically examined to ascertain the presence of nematodes.

A. lineatella

This trial was conducted in a 2-year old non-bearing almond orchard near Cortez, Merced Co., CA, to determine the efficacy of a dormant application of *S. carpocapsae* on overwintering *A. lineatella* larvae. The treated trees were infested with *A. lineatella* overwintering in hibernaculae on the branches. Treatments were applied on 20 January, 1987, with a handgun sprayer and consisted of *S. carpocapsae* at rates of 1.5 and 0.5 million per tree, a conventional dormant spray of diazinon (16.0 liter AI per ha) plus Volck supreme oil (48 liter per ha) and water as a check. The treatments were arranged in a completely randomized design with twenty single tree replicates, each treatment tree separated by an untreated buffer tree. No attempt was made to find dead larvae after the application of the treatments because the hibernacula are difficult to locate in significant quantity. It was decided that a more appropriate and practical evaluation of the efficacy of the nematodes on mortality of overwintering larvae would be to count shoot strikes in the spring. Shoot strikes can be used to evaluate larval control (Summers et al. 1959) since the overwintering larvae which emerge feed on new shoots and bore into them. The shoot tips which are killed as a result of this feeding are relatively easy to identify. Efficacy was evaluated on 27 March, 1987, by counting the total number of shoot tips damaged by *A. lineatella* larvae following emergence of the overwintering generation.

RESULTS AND DISCUSSION

A. transitella

In the Le Grand test, the nematode applications did not statistically increase the mortality of overwintering larvae. Although the mortality rates for the 0.62 and 5.00 million *S. carpocapsae* per tree application seemed to increase larval mortality, there was no significant difference ($P > 0.05$) between rates of application when data were subjected to one way ANOVA. Similarly, no significant relationship was found ($r^2 = 0.014$; $P > 0.05$) when dose/mortality data was subjected to regression analysis (Table 1). No nematodes were found inside the dead larvae.

There was a positive relationship between the number of nematodes applied per tree and the number of nematodes delivered to the mummies ($F = 66.3$; $P < 0.001$) (Table 1). The relationship between dose rate and number of mummies receiving nematodes was also positive, indicating that better coverage is obtained as the number of nematodes applied per tree increases. These are important results as they demonstrate the potential of delivering nematodes to the site of the overwintering larvae using commercially available spraying equipment. Larval mortality in this trial was much lower than that observed by Lindegren et al. (1978) and Agudelo-Silva et al. (1987) for applications at hullsplit (summer). Since there were no differences in larval mortality between treatments and no nematodes were found in the dead larvae, most likely the nematodes did not infect them. This may have been due, at least partially, to temperatures during the time of this study. The low temperature probably impaired the ability of nematodes to move on the mummies to reach the larvae inside them, and even if a nematode ultimately reached a larva, the nematode and its symbiont bacteria may not have lethally infected it. Although the temperature reached 18°C for a few hours during the seven days after the nematodes were applied, most of the time the temperature was too low to allow nematode infection and development. During the seven days following application, temperatures did not exceed 10°C for 60% of the time (101 hours). Development and reproduction of *S. carpocapsae* occurs between 15°C and 27°C (Kaya 1977).

TABLE 1. *STEINERNEMA CARPOCAPSAE* STRAIN ALL JUVENILES APPLIED TO ALMOND TREES FOR CONTROL OF *AMYELOIS TRANSITELLA* LARVAE OVERWINTERING IN MUMMY ALMONDS, 23 FEBRUARY, 1987 (N=5). LARVAL MORTALITY WAS EVALUATED 7 DAYS AFTER TREATMENT.

Mean		Nematodes[2] per Mummy		% Mummies with Nematodes
Number Nematodes per Tree ($\times 10^6$)	Percent Dead[1] Larvae ± SD	Mean %[2] ± SD	Range	
0.00	2.5 (3.6)	0.0 (0)	—	0
0.62	4.0 (6.9)	27.6 (35.6)	12-108	60
1.25	2.7 (6.0)	21.2 (10.0)	12-84	60
2.50	2.6 (2.4)	13.2 (9.8)	12-204	48
5.00	5.3 (11.0)	55.6 (34.6)	12-204	88
10.00	2.0 (2.6)	68.6 (61.1)	12-240	88
20.00	2.1 (3.3)	232.0 (138.0)	36-588	92

[1]Fifty mummy nuts per replicate.
[2]Ten mummy nuts per replicate.

In the Durham test, it was hoped that *Heterorhabditis* sp. strain HL81, a cold-tolerant nematode strain, would prove more effective controlling *A. transitella* during its overwintering period. Acceptable delivery of nematodes to the mummies was achieved with 90 and 82 percent of the mummies for *S. carpocapsae* and *Heterorhabditis* sp. respectively releasing nematodes in the extraction procedure (Lindegren et al 1987) (Table 2). Larval mortality of mummies treated with either nematode species was 2.2 times greater than mortality observed from mummies that were treated with water alone. This difference was statistically significant ($P < 0.05$) and strongly suggests that applications of entomopathogenic nematodes can increase mortality of *A. transitella* in mummy nuts during the dormant period. No nematodes were found inside the dead larvae. Unfortunately, *A. transitella* survival was over 88% for both species. The fact that no nematodes were found inside the dead larvae is not surprising. Most likely, nematodes invaded larvae and killed them, but died before completing development and reproduction. The dead nematodes would have decayed before the dead *A. transitella* larvae were examined. The periods of low temperatures (< 10°C) that occurred between the time of nematode application and the time when the dead larvae were examined (when infection by the nematodes must have taken place) were not conducive for the successful growth of the nematode and its symbiont bacteria (Molyneux, 1986). Successful growth of the symbiont is necessary for nematode development (Poinar & Thomas 1966). The significant ($P < 0.05$) difference between the number of dead larvae found in the mummies treated with nematodes and those treated with water only suggests that the nematodes were responsible for the differences in mortality. It is possible that more cold-tolerant nematode strains than those applied in this trial could increase mortality. However, the increase in larval mortality that could be attributed to nematode treatment in this trial, although important biologically, would not be acceptable relative to conventional controls currently available.

A. lineatella

The difference in the number of *A. lineatella* shoot strikes between the almond trees treated with *S. carpocapsae* during the dormant season (Table 3) at 1.5 million

TABLE 2. *STEINERNEMA CARPOCAPSAE* STRAIN ALL AND *HETERORHABDITIS* SP. STRAIN (HL81) APPLIED AT A RATE OF 51 NEMATODES PER MUMMY FOR CONTROL OF *AMYELOIS TRANSITELLA* LARVAE, 15 DECEMBER 1987.

Treatment	Mean Percent Dead Larvae[1] ± SD	Mean Number Nematodes[2] per Mummy ± SD	Range of Nematodes per Mummy	Percent Mummies with Nematodes
S. carpocapsae	11.8 (4.1)a	26.8 (25.02)	0-126	90
Heterorhabditis sp.	11.6 (2.3)a	19.4 (26.51)	0-162	82
Water check	3.6 (2.8)b	0.0 (0.0)	—	—

[1]Means followed by the same letter are not significantly different (P > 0.05) when compared by Duncan's (1995) new multiple range test.
[2]Ten mummies nuts per replicate.

and 0.5 million per tree and the water check was statistically significant ($P < 0.05$), but the level of control (about 24%) was significantly ($P < 0.05$) less than the level of control (~ 93%) achieved with the conventional dormant treatment of diazinon plus oil (F=34.27; DF=3). These data suggest that *S. carpocapsae* have the ability to penetrate overwintering hibernaculae, and kill the larvae inside them, reducing their overwintering populations and subsequent damage to trees. The hourly temperatures during some of the days when the larvae were exposed to the nematodes was high enough (up to 23°C) to allow nematode movement and larval infection. However, the temperature was low (less than 10°C) for several days during the time that the larvae were exposed to the nematodes, and this probably diminished the growth, reproduction and infectivity of the nematodes (Kaya 1977).

The ability of entomopathogenic nematodes to reduce overwintering *A. transitella* and *A. lineatella* larval populations is not resolved. It was promising that larval mortality was achieved with existing nematode strains under field conditions, and that nematodes could be reliably delivered to mummy almonds in commercial orchards (Le Grand test). However, larval mortality of both *A. transitella* and *A. lineatella*, while significant, was below commercially acceptable levels. It can be hypothesized that if a nematode were available that would remain active in larvae for extended periods of time below 10°C, the level of larval mortality achieved could be greater. It is also pos-

TABLE 3. *STEINERNEMA CARPOCAPSAE* STRAIN ALL APPLIED TO ALMOND TREES FOR CONTROL OF *ANARSIA LINEATELLA* LARVAE OVERWINTERING IN HIBERNACULAE, 20 JANUARY, 1987 (N=20). EFFECT OF TREATMENTS MEASURED AS NUMBER OF LARVAL SHOOT STRIKES PER TREE ON 27 MARCH, 1987.

Treatment	Rate	Mean Shoot Strikes[1] per Tree ± SD
Water check	—	28.4 (10.6)c
S. carpocapsae	1.5×10^7 per tree	22.3 (9.8)b
S. carpocapsae	0.5×10^7 per tree	21.4 (9.3)b
Diazinon + oil	16 l AI per Ha. + 48 L per Ha.	2.1 (2.7)a

[1]Means followed by the same letter are not significantly different (P > 0.05) when compared by Duncan's (1955) new multiple range test.

sible that treatments for both pest species could be delayed until early bloom when warmer conditions would be present, yet overwintering larvae would be present as the treatment target. In order to be commercially feasible at present, the level of larval mortality achieved must reach 70 to 90 percent, and the cost of applying nematodes must be competitive with conventional in-season sprays for *A. transitella*, ($24 to $40 per ha) and dormant sprays for *A. lineatella* ($20 to $56 per ha).

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